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The role of S-mephenytoin hydroxylase (CYP2C19) in the metabolism of the antimalarial biguanides

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The effects of the CYP2C19 substrates, mephenytoin, methsuximide and mephobarbitone on the metabolism of proguanil and chlorproguanil by human liver microsomes were studied. All of the CYP2C19 substrates significantly inhibited ($P < 0.05$) the formation of both cycloguanil and chlorcycloguanil from their parent compounds. In the presence of mephenytoin (50 and 100 μM) the extent of proguanil cyclisation was decreased by 66% and 67% whilst the cyclisation of chlorproguanil was decreased by 51% and 70%, respectively. Methsuximide (50 and 100 μM) inhibited cycloguanil formation by 68% and 77% and chlorcycloguanil formation by 43% and 58%, respectively. In the presence of mephobarbitone (50 and 100 μM) the cyclisation of proguanil and chlorproguanil to their active metabolites was reduced by 24% and 42% and 48% and 63%, respectively. In addition, proguanil and chlorproguanil were shown to be mutual competitive inhibitors of metabolism to their triazine metabolites. In the presence of proguanil (50 and 100 μM) the K_m value for chlorcycloguanil production was increased by 118% and 200%, respectively, with little change in V_{max}. Similarly, chorproguanil (50 μM) increased the K_m for the *in vitro* cyclisation of proguanil by 50% with no alteration in V_{max}. These data suggest that both chlorproguanil and proguanil are metabolised *in vitro* by mephenytoin hydroxylase, CYP2C19.

Keywords mephenytoin hydroxylase CYP2C19 antimalarial proguanil chlorproguanil

Introduction

Proguanil and chlorproguanil are antimalarial prodrugs both of which undergo cytochrome P-450 mediated biotransformation to their active triazine metabolites cycloguanil and chlorcycloguanil, respectively [1]. It has been shown both *in vivo* and *in vitro* that the formation of cycloguanil from proguanil in man is dependent, at least in part, on the polymorphically distributed enzyme mephenytoin hydroxylase, CYP2C19 [2, 3].

Chlorproguanil differs from proguanil by the addition of a chlorine atom at position 3 of the benzene ring. Chlorproguanil cyclisation in the presence of human liver microsomes is inhibited in a competitive manner by mephenytoin [2] suggesting a possible role for CYP2C19 in the metabolism of chlorproguanil. However, the metabolism of chlorproguanil has not been studied *in vivo* in subjects of known mephenytoin hydroxylase phenotype. The *in vivo* metabolism of chlorproguanil has, however, been studied in subjects of known proguanil phenotype [4]. This phenotype has been shown to co-segregate *in vivo* with the mephenytoin hydroxylase polymorphism [3]. Nevertheless, it was found that poor metabolism of proguanil did not correlate directly with poor metabolism of chlorproguanil [4]. The authors suggested that chlorproguanil metabolism was not controlled by the mephenytoin hydroxylase polymorphism. However, these data should be treated with some caution as a single 6 h spot urine sample, taken after a single dose of proguanil was used to establish the metabolic ratio. Earlier work [13] would suggest that this is a less than ideal sample on which to base phenotypic classification.

We have undertaken a series of *in vitro* experiments to determine whether there is any evidence for

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the role of CYP2C19 in the cyclic activation of both chlorproguanil and proguanil. Racemic methsuximide and racemic mephobarbitone have been shown to be competitive inhibitors of CYP2C19 activity as measured by S-mephenytoin 4'-hydroxylation *in vitro* [5]. Mephobarbitone metabolism has also been shown to co-segregate with the mephenytoin hydroxylase polymorphism *in vivo* [6]. We have, therefore, investigated the ability of these substrates of CYP2C19 to inhibit the metabolism of proguanil and chlorproguanil in human liver microsomes. Additionally, based on the assumption that mutual competitive inhibition *in vitro* is an indication of a common metabolic pathway, we have characterised the effects of these biguanides on each others activation *in vitro*.

Methods

Microsomes were prepared by differential centrifugation [7] from a histologically normal human liver with no previous recorded exposure to other drugs. Ethical approval for the study was obtained from the Mersey Regional Health Authority. Microsomal protein [8] and cytochrome P-450 content [9] were determined.

Incubations were performed under conditions previously demonstrated to be linear with respect to time and protein concentration [2]. Each incubate (n = 3) contained microsomal protein (2 mg), drug or drug combination and phosphate buffer (67 mM, pH 7.4) to give a final reaction volume of 1 ml. Reactions were initiated by the addition of NADPH (1 mM) and incubated at 37° C for 30 min. Reactions were terminated by the addition of ice cold methanol (1 ml).

The effect of proguanil (50 μM and 100 μM) on the metabolism of chlorproguanil (12.5, 25, 50, 100, 250, 350 and 500 μM) and the effect of chlorproguanil (50 μM) on the metabolism of proguanil (12.5, 25, 50, 100, 250 and 350 μM) was investigated.

Proguanil and chlorproguanil (50 μM) were also incubated in the absence or presence of racemic mephenytoin (50 and 100 μM), methsuximide (50 and 100 μM) and mephobarbitone (50 and 100 μM). The concentration of proguanil and chlorproguanil used approximated to the K_m for proguanil and chlorproguanil activation as determined previously [10].

All compounds used in these *in vitro* metabolism studies were initially dissolved in methanol due to their poor aqueous solubility and then subsequently diluted with phosphate buffer (67 mM, pH 7.4) such that the final concentration of organic solvent was equal in both control and test incubations and constituted less than 1% (v/v) of the total reaction volume in all cases.

The metabolites cycloguanil and chlorcycloguanil were assayed by h.p.l.c. as described previously [2]. Enzyme kinetic parameters were determined from untransformed data using the ENZPAC program [11]. Statistical analysis was by the Mann-Whitney U test. K_i values were calculated using the Webb equation [12]:

$$K_i = \frac{[I]}{K_m(I)/[K_m(C) - 1]}$$

Where, [I] = concentration of inhibitor (μM), $K_m(I) = K_m$ in the presence of inhibitor (μM) and $K_m(C) = K_m$ in the absence of inhibitor (μM).

Results

Proguanil was found to be a competitive inhibitor of the cyclisation of chlorproguanil to its active metabolite (Table 1). In the presence of proguanil at concentrations of 50 and 100 μM there was an increase in K_m of 118% and 200%, respectively, for the conversion of chlorproguanil to chlorcycloguanil, with little change in V_{max}. Chlorproguanil was found to be a competitive inhibitor of proguanil metabolism to cycloguanil. In the presence of chlorproguanil at a concentration of 50 μM there was an increase of 50% in the K_m for the conversion of proguanil to its active metabolite cycloguanil with no change in V_{max}. In addition, Eadie-Hofstee plots for the formation of cycloguanil and chlorcycloguanil were found to be linear ($r^2 \geq -0.93$) suggestive of a single enzyme mediated reaction. This is also supported by the similar K_i value for proguanil and the K_m value for chlorproguanil cyclisation (Table 1).

The CYP2C19 substrates racemic mephenytoin, methsuximide and mephobarbitone all inhibited the

Table 1 V_{max} and K_m values for the metabolism by human liver microsomes of chlorproguanil in the presence of proguanil and *vice versa*

Substrate	V_{max} *(pmol min^{-1} mg^{-1} protein)*	K_m *(μM)*	K_i *(μM)*	K_i:K_m *(control)*
Proguanil				
Control	12	20	—	—
Chlorproguanil (50 μM)	12	30	33	1.7
Chlorproguanil				
Control	13	22	—	—
Proguanil (50 μM)	11	48	23	1
Proguanil (100 μM)	11	66	33	1.5

metabolic activation of both chlorproguanil to chlorcycloguanil and proguanil to cycloguanil. Inhibition was significant in all cases ($P < 0.05$) with the exception of methsuximide (50 μM) against chlorproguanil cyclisation (Figure 1a). Owing to analytical difficulties the effect of mephobarbitone on proguanil cyclisation (Figure 1b) was determined at a later date than that of mephobarbitone on chlorproguanil cyclisation (Figure 1a). Hence the differences in the control values reported in each case.

Discussion

The results indicate that proguanil and chlorproguanil are mutually competive inhibitors of their metabolism. This, together with the finding of linear Eadie-Hofstee plots for both cycloguanil and chlorcycloguanil formation, would suggest, that these two compounds are metabolised by a single common enzyme, at least *in vitro*. This observation is in accordance with the finding that the metabolism of chlorproguanil can be inhibited competitively by mephenytoin *in vitro* [2]. Further evidence for the involvement of a common enzyme can be obtained from examination of the ratio of K_i relative to control K_m. The observation of K_i to K_m ratios between 1 and 1.7 may indicate a significant role for a common isoenzyme in the metabolism of both proguanil and chlorproguanil.

These data suggest that *in vitro* the metabolism of chlorproguanil is mediated at least in part by the polymorphic mephenytoin hydroxylase (CYP2C19). As a consequence the distribution frequency of the poor metaboliser phenotype of chlorproguanil within ethnic groups is expected to be similar to that observed with proguanil and by implication S-mephenytoin.

We conclude that other substrates of CYP2C19 may therefore have the potential to inhibit the metabolism of chlorproguanil *in vitro*, and more importantly *in vivo*. Caution should therefore be exercised in the use of chlorproguanil as an antimalarial agent within different ethic groups and/or in combination with other substrates or inhibitors of CYP2C19.

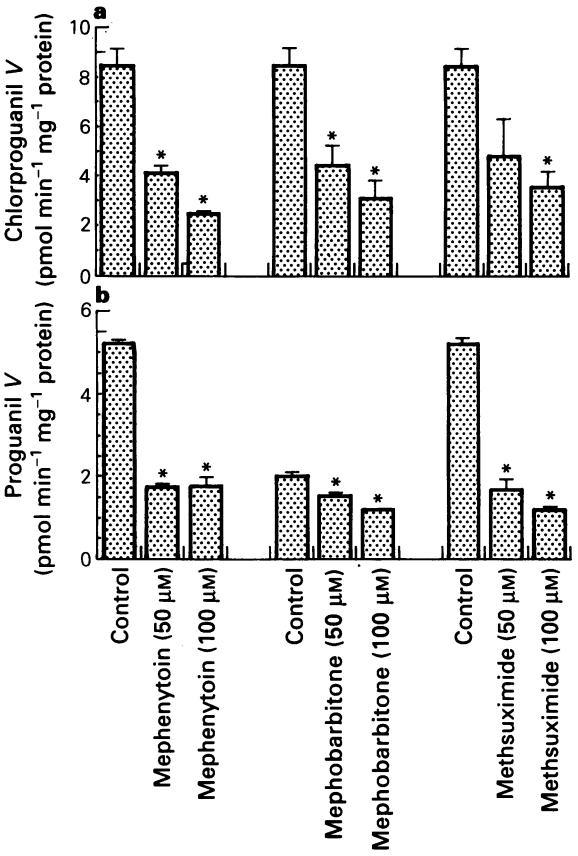

Figure 1 a) Inhibition of chlorproguanil metabolism by the CYP2C19 substrates expressed as mean V (pmol min^{-1} mg^{-1} protein) ± s.d. b) Inhibition of proguanil metabolism by the CYP2C19 substrates expressed as mean V (pmol min^{-1} mg^{-1} protein) ± s.d. *P < 0.05.

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